

Notes on the Biology of *Lycorina triangulifera* Holmgren (Hymenoptera: Ichneumonidae: Lycoriniinae)

MARK R. SHAW

National Museums of Scotland, Chambers Street, Edinburgh EH1 1JF, U.K.

Abstract.—Host searching behaviour, oviposition and the ovarian egg of the koinobiont ichneumonid *Lycorina triangulifera* are described. Oviposition attacks were observed on two species of leaf spinning tortricid larvae, the ovipositor being inserted into the host's anus. The ovarian egg does not have the sinuous and leech-like characteristics described and figured by Iwata (1958). Rather, its elongate oval shape is unremarkable, but it carries a small anchoring device at its (presumed) caudal end. This strongly suggests that the egg is laid externally with respect to the host cuticle. One adult was reared from the experimental tortricid host *Acleris schalleriana*, but observations were not close enough to ascertain whether the development of the larva was endoparasitic or ectoparasitic. Nevertheless, it seems probable that *Lycorina* is essentially a koinobiont ectoparasitoid with an unusual egg placement.

This paper presents an opportunistic study on aspects of the biology of an ichneumonid, *Lycorina triangulifera* Holmgren, belonging to a small subfamily, Lycoriniinae, whose systematic position within the Ichneumonidae remains uncertain. Direct observations on the biology of the Lycoriniinae have not previously been published (but see Coronado-Rivera et al. 2004).

The subfamily is widely distributed in the world but according to Yu and Horstmann (1997) it contains fewer than 30 valid described species, all classified in the genus *Lycorina* Holmgren, although Townes (1970) had recognised three closely related genera in the subfamily (*Lycorina*, *Gonioglyphus* Seyrig and *Toxophoroides* Viereck), each distributed in a different part of the world. Although some credible rearing records exist (from various "microlepidoptera," traceable via Yu & Horstmann 1997), no details beyond that of the host's name have been published (but see also Coronado-Rivera et al. 2004). One species, *L. triangulifera*, occurs widely in the Palaearctic region and has very occasionally been collected in southern Eng-

land in wooded habitats. It has been widely recorded as reared from Tortricidae, Yponomeutidae, Pyralidae and Gelechiidae. Unfortunately the relatively large number of literature citations of these hosts generally fail to make it clear whether the record given is a reiteration of a previous (but unreferenced) citation, or a new finding: none that I have seen is detailed or authoritative enough to be worth citing here, but references to arboreal Tortricidae seem the most diverse and therefore perhaps the most credible. There is also a welter of references to the cerambycid beetle *Saperda populnea* (Linnaeus) as host, but again they may all relate to a single supposed occasion which is in any case likely to have been erroneous. Gauld (1997) briefly discusses the host records of other species, emphasising Pyralidae and Tortricidae, but Lycoriniinae remains one of the few subfamilies of Ichneumonidae for which practically nothing is known about its developmental biology (but see Coronado-Rivera et al. 2004).

Cushman & Rohwer (1920) first proposed independent status for Lycoriniinae on the basis of its morphological isolation.

and succeeding authors (e.g. Perkins 1959, Townes 1970) have maintained that view. More recently, molecular phylogenies (D. L. J. Quicke et al. pers. comm.) have continued to emphasise its distinctiveness. According to D. L. J. Quicke (pers. comm.) the D2 region of the 28S rDNA gene is on a long branch: in parsimony analyses with all other subfamilies *Lycorina* tends to associate either with Anomaloninae or with Hybrizontinae; however, members of both also exhibit long branches, and therefore such placements are best regarded as artefactual. The detail of the ovipositor structure, in which the upper valve is divided in two with a separate piece linking them, is similar to that seen in some of the possibly basal "ophioniformes" (e.g. Stilbopinae, Cremastinae, Banchinae, Phytodietini, Idiogrammatini and some Phru-dinae), suggesting another possible affinity (Quicke et al. 1994, Quicke pers. comm.), although single sections of such structures are difficult to interpret with confidence. Further information illustrating the uncertain phylogenetic position of Lycorininae is summarised by Coronado-Rivera et al. (2004).

MATERIALS AND METHODS

In view of its previously known distribution in Britain, it was a great surprise when, at about 23.30 BST on 29.vi.2003, a female specimen of *L. triangulifera* came to a 160w MV-Tungsten blended light over a sheet run (with a generator) at Wood of Brae, on the Black Isle (Cromarty), in N. E. Scotland (Grid Ref. NH 692628). The habitat consisted of a ca 10m strip of boggy heath between a vehicle track (with open heathland on the other side) and birch-dominated thicket woodland, with stunted (browsed) bushes of *Betula*, *Alnus* and *Salix* near to the light. It is not an unusual habitat in Scotland, though the presence of several species of orchid and other ground flora suggested that it had been moderately stable. The female was kept alive, fed *ad libitum* on 1:3 honey:water,

pure honey and pure water (all of which were accepted, sparingly, at different times) in the 7.5 × 2.5cm corked glass tubes in which she was always kept and offered potential hosts. The simplest aim was to try to discover its oviposition biology, but unfortunately most of this necessarily happened during a holiday in Belgium and E. France (8–26.vii.2003), when the frequent travelling as well as sometimes high temperatures made it difficult to keep the female in good condition (and may have hastened her death on 24.vii.2003). It was also difficult to make as many or as detailed observations as would have been desirable, and neither suitable photographic nor microscopy equipment was to hand. Further, only wild-collected hosts were available to offer to the female, and the supply of these was sparing. Possibly parasitised hosts were reared singly in 7.5 × 2.5cm corked glass tubes, with absorbent tissue paper pressed into the bottom (cf. Shaw 1997). For most of the period until 26.vii.2003 they were under essentially indoor conditions, but subsequently all livestock was kept in a fully shaded and well ventilated outdoor shed.

A further female, collected in France: Dordogne, St Alvère, by Malaise trap 13–25.vi.2002 (*R.R.Askew*) was stored in ca 60% ethanol until 15.ii.2004, when its ovarian eggs were examined by dissection.

All adults of *Lycorina triangulifera* mentioned in this work are deposited in the National Museums of Scotland.

RESULTS AND DISCUSSION

Host Acceptance Trials

In early trials (2–6.vii.2003) the general behaviour of the female *L. triangulifera* towards Lepidoptera larvae and their faeces was found to be as follows: naked hosts (both "macro" and "microlepidoptera") were never of the slightest interest; faecal pellets, if small, offered alone often elicit-

Table 1. Responses of adult *Lycorina triangulifera* (1 ♀) to various "microlepidoptera" larvae in leaf spinings. (No ovipositions occurred).

Larva	Instar	Leaf spinning	Faeces attractive?	Probing?
<i>Agonopterix</i> sp. (Oecophoridae)	Final	<i>Angelica</i>	No	No
<i>Diurnea fagella</i> (Dennis & Schiffermüller) (Oecophoridae)	Early	<i>Fagus</i>	—	Yes
2 × <i>Hypatima rhomboidella</i> (Linnaeus) (Gelechiidae)*	Final	<i>Betula</i>	No	No
<i>Ancylis mitterbacheriana</i> (Dennis & Schiffermüller) (Tortricidae)	Penultimate	<i>Quercus</i>	—	Yes
Indet. (Tortricidae)	Penultimate	<i>Crataegus</i>	Yes	Yes
Indet. (Tortricidae)	Penultimate	<i>Rosa</i>	Yes	No
Indet. (Tortricidae)	Final	<i>Salix</i>	No	No
<i>Udea</i> sp. (Pyralidae)	Final	<i>Rubus</i>	Yes	No
Indet., absent	—	<i>Acer</i>	—	Yes**
Indet., absent	—	<i>Salix</i>	—	Yes**

* Both turned out to be already parasitised by *Meteorus pulchricornis* (Wesmael) (Braconidae).

** Vacated roll probed for more than 3 minutes.

ed intense antennation and simultaneous (or closely subsequent) probing with the ovipositor, a little forward of vertically, against the glass of the tube (faeces of young Geometridae and Notodontidae feeding on *Betula* were the most attractive tested, but comparisons were few); leaf spinings containing "microlepidoptera" larvae often elicited probing responses. The faecal pellets of larval sawflies (Hymenoptera) in the families Argidae (on *Salix*) and Tenthredinidae (on *Betula* and *Spiraea*) were as unattractive as the larvae that produced them. Various wild-collected "microlepidoptera" larvae concealed in leaf spinings were offered *in situ* in the period 2–23.vii.2003 but not parasitised, although in some cases they elicited probing responses. The results of these trials, which mostly involved single examples of the Lepidoptera species, are expressed in Table 1. Many of these larvae were already in their final instars when offered and, in view of later experiments, it seems possible that at least some may have been too far advanced to be acceptable.

The remainder of the observations (in the period 12–23.vii.2003) were made on tortricid species into which ovipositions apparently occurred (*Ancylis apicella* (Den-

nis & Schiffermüller) (Olethreutinae) in *Frangula alnus* Miller and *Acleris schalleriana* (Linnaeus) (Tortricinae) in *Viburnum opulus* Linnaeus spinings); potential hosts that could be collected in small numbers where my wife and I were holidaying, allowing at least a bit of continuity and control. Although three apparent ovipositions were observed in *A. apicella* (and a further three larvae may have been oviposited into, as each had to be left unobserved while the parasitoid was probing its leaf roll) only adult moths resulted from all exposed hosts (all but one completed their development in 2003 rather than entering diapause as a fully fed larva). In contrast, the only *A. schalleriana* into which oviposition took place resulted in progeny. No difference was seen in the way the female *Lycorina triangulifera* behaved towards these two tortricids, which both seemed fully acceptable to her despite the apparent difference in developmental success. The following descriptions of probing and oviposition behaviour are based on these hosts. The host individuals concerned were all associated with single spun leaves, in which they were well established. Some of the *Ancylis apicella* larvae offered were well grown in their final

instar but most (and all *Acleris schalleriana*) were in earlier (second to penultimate) instars, and ovipositions were only seen in hosts within that range.

Probing Behaviour

Leaf rolls (or folds—"roll" is used to cover both) were rapidly accepted and climbed onto following very brief antennation. Probing with the ovipositor followed immediately, interspersed with much antennation of the substrate. The ovipositor was rapidly and repeatedly plunged deeply into the roll, showing no favour for windowed or holed areas; the female made these successive insertions as she moved along the roll, giving the impression that she was following detected movements of the host. This impression was enhanced by her repeated, sudden and rapid turns (often 180°) and subsequent methodical probings along a new line. However, some of the rolls she investigated were sufficiently windowed for the (unchanging) position of the host larvae to be seen, and other rolls in fact contained no larvae, so these sudden changes of tack in the parasitoid's searching appear to be completely independent of host behaviour, and at least the initial contact with the host seemed therefore to depend largely on chance. Although the parasitoid's probing appeared to be extremely meticulous and thorough, on several occasions she was observed to spend more than 20 minutes continuously probing a roll but failing to contact the host within. Rolls in which this happened were eventually abandoned, at least for a time, after about 25 minutes. The vigour with which she plunged the ovipositor through intact leaf tissue, with no apparent hesitation for site selection, and the number of times she seemed to have to do it in order to parasitize a single host, may go some way towards explaining the very robust and toothed ovipositor tip of *Lycorina* and also the relatively large subgenital plate (hypopygium) that supports its shaft. The

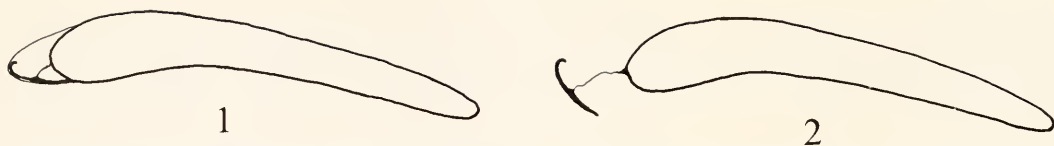
strongly pectinate claws would presumably provide the grip needed to accomplish such sudden turns. The high time investment that appeared to be necessary to parasitise a host is surprising in view of the rather high number of ovarian eggs noted by Coronado-Rivera et al. (2004), and may suggest that females live for quite a long time.

Oviposition Behaviour

Altogether five successful contacts with a host larva were seen, in which it appears the host was extremely rapidly injected with a venom as contact was made, resulting in a temporary and only partial loss of movement. In one case the host immediately wriggled completely out of the end of the roll unnoticed by the parasitoid and was not rediscovered in its subsequent torpor, but in the other four what looked like successful subsequent ovipositions were seen.

In two cases the host (one each of *Acylis apicella* and *Acleris schalleriana*) wriggled head first out of the roll before becoming subdued, and was rapidly grabbed by the parasitoid which held it in her front and middle pairs of legs (orientated head to head) while the ovipositor tip was inserted carefully into the host's anus, and held there for ca 2–2.5 minutes. The ovipositor was then withdrawn, the host released, and the adult parasitoid left the scene. Within a few minutes the host had recovered full mobility and re-entered its roll. There was no discernible difference in the parasitoid's behaviour in these two cases and, although only one of the hosts (*A. schalleriana*) subsequently produced a parasitoid, an egg appeared to have been laid in both cases.

The other two presumed ovipositions (into *A. apicella*) took place through the leaf tissue with the host larva still inside the roll, and in only one case could the host be seen clearly enough for the site of oviposition to be established: again into the anus. In one case the host larva half-



Figs 1–2. Mature ovarian egg of *Lycorina triangulifera*. 1. As it appears in the oviduct. 2. Freed from the apparently membranous tissue seen in Fig. 1.

emerged head first from the roll but was driven back inside by the parasitoid (using its front legs and possibly antennae to hit the larva), and in the other the larva became subdued without exposing itself. The apparent ovipositions took ca 1.5 and ca 2.5 minutes. Although only a proportion of ovipositions appear to involve grasping the host, the strongly pectinate claws of *Lycorina* may also help it to do this.

The Ovarian Egg and Subsequent Development

Iwata (1958) described and figured the ovarian egg of *Lycorina triangulifera* as sinuous and leech-like, completely unlike that of any other known group of Ichneumonidae. Iwata's work does not make clear how the *Lycorina* egg was obtained or prepared, but this peculiarity of the egg of *Lycorina* has been widely cited and is an important part of the enigma surrounding its biology.

The female specimen of *L. triangulifera* from France which was preserved in ca 60% ethanol for about 20 months was in a slightly distended condition when it was dissected. The elongate, white, densely packed ovarian eggs measured 0.62 mm in length and were apparently in good condition. Projecting from the side of the broad leading end (presumably the caudal end: cf. Quicke 1997) of each was a fine brown linear structure, aligned with the long axis of the egg and enclosed in what appeared to be a weak membrane (Fig. 1). When freed from this, the linear structure could be seen to be a thin sclerotised bar, sharply curved at one end (the end that

projects in Fig. 1), and attached from its midlength to a protuberance at the extreme end of the egg by a fine flexible strand (Fig. 2). Coronado-Rivera et al. (2004) have independently discovered and described closely similar devices on the eggs of two species of *Lycorina* from Costa Rica.

Egg placement via the host's anus might in principle be either subcutaneous (i.e. internal) or external with respect to the body of the host, as the lining of the hind gut of caterpillars is part of their cuticular tissue (and sloughed with the rest of the cuticle at ecdysis). At least two other species of Ichneumonidae are known to oviposit into their host's anus. One, *Chorinaeus funebris* (Gravenhorst) (Metopiinae), oviposits into the hind gut and the ensuing larva crosses the gut wall to become an endoparasitoid for the rest of its development (Aeschlimann 1974). In the other case, *Eromenus calcator* (Müller) (Tryphoninae), the egg is fastened to the inside wall of the hind gut and larval development is ectoparasitic throughout (Zinnert 1969). With its very clear anchoring device, it seems probable that the egg of *Lycorina* is positioned externally to the body of the host, albeit in a concealed site, and that *Lycorina* may be essentially an ectoparasitoid. However, until definitive observations can be undertaken the more remote possibility that oviposition is subcutaneous cannot be completely ruled out.

Unfortunately the progress of experimental hosts could not be followed closely enough to provide clear evidence for the site of larval development. The single experimental host (*Acleris schalleriana*) from

which a parasitoid was reared was parasitised (as a probably early third instar larva) on 20.vii.2003, and became prepupal about 8.viii.2003, soon after which its cocoon was opened and the prepupa briefly inspected (but from one side only), without anything unusual being noted. The host cocoon was opened again on 14.viii.2003, by which time the *Lycorina* larva had become fully fed, pushed the host's prepupal cuticular remains entirely to one end of the host's cocoon, and spun its own translucent, very fine and frail membranous cocoon—indistinguishable to me from that typical of *Glypta* (Ichneumonidae: Banchinae) species—in the space that had been occupied by the host prepupa. A slightly deformed female *Lycorina triangulifera* emerged on 6.ix.2003, having chewed a rough hole through the side of the cocoon near its apex and also the adjoining dry leaf tissue. It was fed *ad libitum* on 1:3 honey:water and kept under outdoor shade conditions of daylight and temperature, but its behaviour did not suggest that it would attempt to hibernate and it died on 1.x.2003.

While the above observations demonstrate that *Lycorina* is a koinobiont killing the host as a prepupa, unfortunately they are not complete enough to ascertain how the larva develops. As noted by Coronado-Rivera et al. (2004), the final instar larva has some characters usually seen in endoparasitoids, but also denticulate mandibles (Finlayson 1976, Short 1978, Chao 1980) like those of several groups of ectoparasitic Ichneumonoidea but unlike those of fully endoparasitic ones, suggesting that *Lycorina* may develop as an ectoparasitoid, at least in its final feeding phase. This, indeed, has been confirmed by Coronado-Rivera et al. (2004). Although a switch from endophagy to ectophagy is known to occur widely in non-cyclostome Braconidae (Shaw and Huddleston 1991), in Ichneumonidae it has been believed to occur only in the Eucerotinae (Tripp 1961), whose biology is remarkable for its ex-

treme specialisations (though in fact actual development during the parasitoid's internal phase has not been clearly demonstrated for Eucerotinae). Thus it would be surprising if a genuine switch from endophagy to ectophagy occurred in Lycorininae, and a more parsimonious conclusion would be that, in the strict sense of not being subcutaneous, it is probably an external parasitoid throughout, however long it may take for the larva to emerge from the host's anus. It is frustrating in the extreme that the interventions made were ill-timed to elucidate this crucial aspect of its biology.

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